Detecting pathogenic microorganisms in prod. for human consumption - by eliciting enzyme prodn. by the microorganism and detection with a fluorogenic substrate

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• **Abstract :**

WO8904372 A (A) A process for assaying a dilute concn. of living pathogenic microorganisms in a sample of prod. for human consumption comprises (a) contacting the microorganisms of the sample with an actuating medium comprising (1) a nutrient which is capable of supporting metabolism of the microorganisms, (2) a prodn. agent (I) which is capable of inducing the prodn. of an enzyme in the microorganisms, (3) a fluorogenic substrate (II) which is capable of reacting with the enzyme to release the fluorescent portion and (4) a permeability agent (III) which is capable of increasing the permeability of the microorganism to the enzyme, (II) or both, (b) incubating the mixt. in an environment which allows (1) metabolism of the microorganisms, (2) prodn. of the enzyme, (3) contacting of the enzyme with (II) and (4) release of the fluorescent portion of the fluorescent conjugate, (c) irradiating, at intervals during the incubation period, the fluorescent portion with light of a wavelength sufficiently close to that of an excitation wavelength characteristic of the fluorescent portion, and sufficiently intense, as to cause the fluorescent portion to fluoresce, (d) measuring, at such intervals, the amt. of emitted fluorescence, (e) calculating the velocity of emitted fluorescence, and (f) determining the concn. of microorganisms from such velocity and a preestablished velocity-to-concn. correlation schedule.

USE - The methods are used to determine total coliforms (TC), fecal coliforms (FC) or mesotrophic heterotrophs in e.g. drinking water, bathing water, food or food prepn. equipment. (Dwg.0/8)

EP-386051 B A rapid process for assaying an original liquid or liquefied sample for live coliform microorganisms, the sample for possible human consumption, the process comprising: concentrating the microorganisms on a filter, contacting the microorganisms with an actuating medium, incubate and irradiate the mixture, characterised in (a) collecting a known volume of said original liquid or liquefied sample; (b) concentrating the live microorganisms upon a filter; (c) placing the filter and the microorganisms held thereupon in an actuating medium in an incubation container, the actuating medium comprising: (1) a nutrient for supporting metabolism of the live coliform microorganisms; (2) a production agent for inducing the production of an enzyme in said live coliform microorganisms while they are metabolising; (3) a fluorogenic substrate for reacting with the enzyme to release 4-methylum-beliferone thereof; and (4) sodium lauryl sulphate effective in enhancing fluorescence; (d) incubating the mixt. for a brief period of time, less than one hour and insufficient to permit substantial reproduction of the live coliform microorganisms, under temp. conditions which allow during the brief period of incubation; (1) metabolism of the live coliform microorganisms, (2) production of the enzyme; (3) reaction of the enzyme (whether inside or outside the live coliform microorganisms) with the fluorogenic substrate; and, (4) production of 4-methylumbeliferone of the fluorogenic substrate; (e) irradiating, at intervals during the incubation period, the 4-methylumbeliferone with light of a wavelength sufficiently close to that of an excitation wavelength characteristic of the fluorescent portion, and sufficiently intense, as to cause the 4-methylumbeliferone to fluoresce; (f) measuring at such intervals, the amount of emitted fluorescence; (g) calculating a rate of change of emitted fluorescence; and (h) determining the concentration of the live coliform microorganisms in the original sample from the rate of change and a preestablished rate of change-to-concentration correlation schedule. (Dwg.0/3)

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EP-574977 B A process for assaying living coliform bacteria in a liquid or liquefied sample of product for human consumption having a bacterial concentration as low as one bacterium per 100 millilitres, and in a time of at least six hours, the process comprising: concentrating the microorganisms on a filter, contacting the microorganisms with an actuating medium incubating and irradiating the mixture, characterised in (a) concentrating the bacteria upon a filter having pores sufficiently small to retain the bacteria; (b) placing the filter and bacteria held thereby against a culture medium in a container, the culture medium comprising: (1) a nutrient for supporting metabolism and reproduction of the bacteria, (2) a production agent for inducing the production of an enzyme in said bacteria when the bacteria are metabolizing, (3) a fluorogenic substrate for reacting with the enzyme to release 4-methylumbeliferone from the fluorogenic substrate for reacting with the enzyme to release 4-methylumbelliferone from the fluorogenic substrate, and (4) sodium lauryl sulphate effective in enhancing fluorescence; (c) incubating the culture medium, filter, and bacteria under conditions which allow, during the period of incubation: (1) metabolism and reproduction of the bacteria, (2) production of the enzyme, (3) reaction of the enzyme with the fluorogenic substrate, and (4) release of sufficient of 4-methylumbeliferone from the fluorogenic substrate from each single bacterium and its descendants to form a visible microcolony under fluorescent conditions; (d) irradiating the microcolonies with light of a wavelength sufficiently close to that of an excitation wavelength characteristic of the fluorescent portion, and sufficiently intense, as to cause the microcolonies to fluoresce; and (e) counting the number of fluorescent microcolonies. (Dwg.0/5)

US5292644 A Analysing a natural population of live coliform bacteria in an original liq. or liquefied sample for human consumption comprises contacting the microorganisms with a methyl umbelliferone substrate. Substrate is hydrolysed into methyl umbelliferone by an enzyme secreted by the bacteria, and hydrolysis is accelerated by sodium lauryl sulphate, which renders the microorganisms move permeable to the substrate and/or the enzyme. Methyl umbelliferone is detected by fluorescence in soln. or an agar medium.

ADVANTAGE - Method is rapid. (Dwg.0/5)

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• **Publication data** :

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